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Efficient Quantification of HIV-1 in Heparin Plasma Spiked with Cultured HIV-1 by the Roche Cobas TaqMan and Abbott RealTime HIV-1 Tests

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The current automated real-time HIV-1 viral load assays, the Roche Cobas AmpliPrep/Cobas TaqMan test and the Abbott RealTime test, are FDA cleared for use with EDTA plasma. We show that both real-time reverse transcription-PCR (RT-PCR) tests reliably quantify HIV-1 RNA in heparin plasma specimens spiked with HIV-1 isolate MN.

Many HIV-1 cohort studies began archiving specimens in the mid-1980s before nucleic acid tests were available for viral quantification (13). The majority of clinical specimens archived prior to 1996 were collected in heparin, a highly sulfated glycosaminoglycan used to prevent anticoagulation of blood, which permitted purification of peripheral blood mononuclear cells (PBMC) and plasma for cellular and serological testing. The high negative charge density of heparin results in protein binding and in the case of RNA and DNA polymerases prevents enzymatic action at their respective nucleic acid binding sites (4, 6–8), thereby inhibiting PCRs. While viral burden determination of heparin samples from early time points is critical to many cohort study analyses (5, 10, 15), such data cannot be generated using earlier versions of FDA-cleared viral load assays following approved procedures.

First- and second-generation HIV-1 viral load assays used chaotropic salts (guanidine thiocyanate) to release viral RNA which was concentrated by ethanol precipitation, resulting in a crude RNA extract containing heparin. The Boom silica extraction method has been used routinely in viral load determination of heparin plasma specimens and as a modification of the Roche Amplicor HIV-1 Monitor test (3, 9) to overcome heparin inhibition (2, 16). This method is technically demanding and utilizes acetone to remove liquid from the glass beads employed. Another procedure used to remove heparin was treatment with heparinase, which resulted in HIV-1 RNA values that were lower than in EDTA plasma (3, 6, 7, 12, 14). Glass membrane column methods based upon the silica procedure (Qiagen viral RNA extraction kit; Qiagen Inc., Valencia, CA) were not recommended by the manufacturer for use on heparin plasma samples and were found to be inadequate at the removal of heparin (L. L. Jagodzinski, unpublished data). Organic extraction methods remove heparin, permitting nucleic acid amplification, but are less practical in clinical diagnostic laboratories. The FDA-cleared automated extraction systems employed by the Roche and Abbott real-time platforms use either silica magnetic beads or ferrous iron, respectively, to purify HIV-1 RNA. These methods would be expected to yield RNA free of heparin based upon their extraction chemistries. Neither manufacturer's package insert asserts performance claims for heparinized plasma (1, 11).

The performance of the two FDA-cleared real-time HIV-1 viral load assays, Roche Cobas AmpliPrep/Cobas TaqMan HIV-1 (Roche TaqMan) and Abbott RealTime HIV-1 (Abbott RealTime), were assessed for use on archived specimens collected in heparin. Although our study emphasis is on heparin plasma samples, other commonly used anticoagulants were also assessed. The Roche Amplicor HIV-1 Monitor Test v1.5 (Roche 1.5) was selected as the reference assay in this study. The objectives of this study were as follows: (i) to determine whether the extraction methods used in each assay sufficiently eliminated heparin-associated inhibition, permitting accurate quantification of HIV-1 RNA; and (ii) to assess the impact of other plasma matrices on quantification of HIV-1 RNA.

Plasma units from four healthy donors collected in each of four different matrices (EDTA, acid-citrate-dextrose [ACD], sodium citrate, and sodium heparin) were purchased from Biological Specialty Corporation (Colmar, PA). All units were confirmed to be HIV-1 negative based upon HIV-1 enzyme immune assay (EIA) and HIV RNA tests, Roche 1.5 and the Abbott RealTime HIV-1 test. A modified version of the Roche 1.5 assay (silica-Roche 1.5) using silica extraction reagents purchased from bioMérieux, Inc. (NucliSens lysis and extraction kits) (Durham, NC) was used for assessment of heparin plasma for quantification of HIV-1 RNA (3).

The HIV-1 RNA concentration of a high-titer HIV stock (subtype B; isolate MN) was determined by testing 1,000- and 10,000-fold serial dilutions in the Abbott RealTime assay. A mean of three measurements was used to determine the viral stock concentration. Working stocks of 1E+07 copies/ml HIV-1 were prepared in each matrix. Each working stock was subjected to 10-fold serial dilutions into appropriate plasma matrices to generate HIV-1 specimen panels with concentrations ranging from 1E+06 to 1E+02 HIV-1 RNA copies/ml. Panel aliquots of 1.2 ml were stored at less than −70°C. Each concentration was tested in singleton or in duplicate over at least two test runs in the real-time RT-PCR assays and in triplicate within a single test run in the Roche 1.5 test following the manufacturers' recommended proce-

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TABLE 1 Performance analysis of HIV-1-spiked EDTA and heparin plasma samples in three HIV-1 viral load tests[a]

Nominal no. of HIV-1 $\log_{10}$ copies/ml	Roche Amplicor HIV-1 Monitor test v1.5[b]							Roche Cobas TaqMan HIV-1 test				Abbott RealTime HIV-1 test			
	EDTA		EDTA with silica		Heparin with silica			EDTA		Heparin[d]		EDTA		Heparin	
	Mean	SD	Mean	SD	Mean	SD	Heparin[c]	Mean	SD	Mean	SD	Mean	SD	Mean	SD
6.46	NT	NT	NT	NT	NT	NT	INH	6.49	0.09	6.42	0.32	6.36	0.04	6.35	0.07
5.46	5.42	0.15	5.52	0.08	5.34	0.21	INH	5.41	0.06	5.28	0.13	5.41	0.02	5.38	0.05
4.46	4.47	0.15	4.36	0.18	4.26	0.20	INH	4.47	0.06	R	R	4.37	0.01	4.33	0.03
3.76	3.97	0.13	3.66	0.14	3.61	0.08	INH	3.49	0.04	3.46	0.09	3.62	0.12	3.60	0.06
3.06	3.02	0.14	3.35	0.38	2.80	0.10	INH	2.88	0.16	2.68	0.06	2.93	0.04	2.88	0.03
2.76	2.68	0.55	2.73	0.40	2.90	0.27	INH	2.60	0.16	2.57	0.08	2.64	0.09	2.62	0.07

[a] Analysis of viral load results for mean HIV-1 RNA concentration in $\log_{10}$ copies/ml and reproducibility.
[b] NT, not tested. The specimens at this concentration were not tested in the Roche 1.5 assay, since the target concentration was above the performance range of the assay.
[c] INH, inhibition. HIV-1-spiked heparin specimens failed to provide any absorbance values in the Roche 1.5 assay due to the carryover of heparin in the crude viral RNA, resulting in inhibition of the RT-PCR amplification reactions.
[d] R, removed. This concentration was removed from the analysis for the heparin samples in the Roche Cobas TaqMan test due to an insufficient number of results.

dures. Additionally, HIV-1-spiked heparin and EDTA plasma samples were also tested using the silica-Roche 1.5 method.

HIV-1 RNA assays employ either a quantitation standard (QS) or an internal control (IC) for use in determination of the viral RNA concentration and in the assessment of extraction and/or RT-PCR amplification efficiency. The Roche 1.5 test is an end-point analysis assay requiring 5-fold serial dilutions to obtain the absorbance values for the QS and the HIV-1 targets. A valid test result requires a QS absorbance value of greater than 0.3 OD_{450} unit (OD_{450} stands for optical density at 450 nm) at 1- or 5-fold dilutions. The concentration of the viral RNA is calculated using the absorbance values of both the HIV-1 target and the QS. The Roche Cobas TaqMan and Abbott RealTime assay measurements occur as the amplification proceeds. Performance ranges for real-time assays are based upon cycle threshold (C_T) values that represent the amplification cycle at which the target fluorescent signal is above a specified background level. The Roche Cobas TaqMan assay uses the QS value and kit-specific calibration coefficients to calculate the HIV-1 RNA concentration and as a monitor of assay performance. The Abbott RealTime assay uses the IC value only as a monitor of assay performance. Amplification failure for an individual specimen may be due to poor yield during extraction or RT-PCR inhibition. Repeat testing of a failed specimen that results in a subsequent amplification failure indicates the presence of an inhibitory substance, whereas a valid test result is indicative of an extraction failure in the first test run.

HIV-1 RNA concentrations were determined for HIV-1-spiked EDTA plasma specimens using the standard Roche 1.5 test and the modified silica-Roche 1.5 for heparin plasma specimens (Table 1). HIV-1 concentrations were equivalent for HIV-1-spiked EDTA plasma specimens tested by the Roche 1.5 assay following the recommended procedures and by the modified silica-Roche 1.5 assay. As expected, HIV-1 RNA extracted from the heparin plasma specimens did not amplify in the Roche 1.5 test (standard format) as evidenced by a lack of absorbance for the QS and the HIV-1 analyte in the assay. In contrast, no inhibition by heparin was observed in the modified silica-Roche 1.5 assay and in either one of the real-time HIV-1 RT-PCR assays. Cycle threshold values were within assay-specified ranges for the QS in the Roche Cobas TaqMan assay and the IC in the Abbott RealTime assay. The results of linear regression analyses of the combined viral load test results for each matrix in each of the assays are shown in Fig. 1. HIV-1 RNA concentrations determined for HIV-1-spiked EDTA and heparin plasma samples were not significantly different ($P = 0.9965$ by one-way analysis of variance [ANOVA]). Viral RNA concentrations correlated significantly ($r^2 = 0.9780$ to 0.9995 and $P < 0.002$ for Roche 1.5 and $P < 0.0001$ for the Roche TaqMan and Abbott RealTime by two-tailed Pearson test). Performance was linear across the range of concentrations tested. The Roche 1.5 assay demonstrated higher variability as evidenced by the higher percent coefficient of variance (CV) and a greater standard deviation (SD) than the automated platforms with the greatest variability occurring in HIV-1 RNA concentrations under 10,000 copies/ml (4 $\log_{10}$ units [Table 1]). All three assays demonstrated less variability at HIV-1 RNA values above 10,000 copies/ml. Similar performance data were obtained for HIV-1 spiked into sodium citrate and ACD plasma matrices with all HIV-1-spiked matrices, demonstrating linear regression curves that were parallel and superimposed with those for EDTA and heparin plasma samples (data not shown). The Roche Cobas TaqMan and Abbott Real-

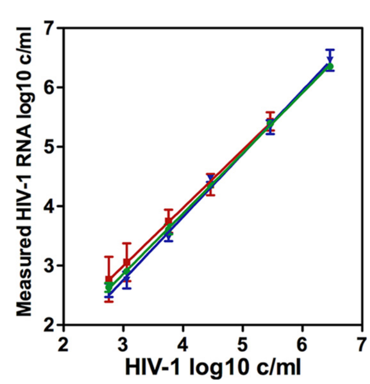

FIG 1 Performance profiles for HIV-1 viral load assays with HIV-1-spiked EDTA and heparin plasma samples. EDTA and heparin plasma viral load test results were combined for each assay and subjected to linear regression analysis with determination of the mean values and performance ranges. The Roche 1.5 assay is the reference assay in this study. The Roche 1.5 assay results are shown as red squares. The Roche TaqMan results are shown as blue inverted triangles, and the Abbott RealTime assay results are shown as green circles. The number of HIV-1 $\log_{10}$ copies per milliliter are shown.

Time assay viral load results for HIV-1-spiked EDTA and heparin plasma samples were comparable and demonstrated improved reproducibility compared to the manual Roche 1.5 test as evident by the range of standard deviations: 0.08 to 0.55 for the Roche 1.5 assay, 0.04 to 0.32 for the Roche TaqMan assay, and 0.01 to 0.12 for the Abbott RealTime assay (Table 1).

Quantitation of HIV-1 RNA in heparin plasma specimens requires modification of FDA-approved procedures in first- and second-generation HIV-1 viral load assays. While extraction of HIV-1 RNA from heparin plasma specimens using the Boom procedure allows for reliable quantification in the Roche 1.5 test, the procedure is tedious and labor-intensive. Additionally, the Roche 1.5 assay will no longer be available on the U.S. market in the early summer of 2012, and laboratories will have to shift to the next-generation real-time RT-PCR assays. This study clearly demonstrates that the Roche Cobas TaqMan and Abbott RealTime tests efficiently remove the inhibition effect of heparin and therefore can be used to generate accurate and reliable viral burden data from archived heparin plasma specimens without assay modification. The linear performance of both assays also permits accurate testing of diluted specimens, which is particularly important when assessing low-volume archival specimens.

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REFERENCES

1. **Abbott Molecular Inc.** 2009. Abbott RealTi*me* HIV-1 assay package insert. REF 6L18. 51-602146/R4. Abbott Molecular Inc., Des Plaines, IL.
2. **Boom R, et al.** 1990. Rapid and simple method for purification of nucleic acids. J. Clin. Microbiol. **28**:495–503.
3. **Dickover RE, et al.** 1998. Optimization of specimen-handling procedures for accurate quantitation of levels of human immunodeficiency virus RNA in plasma by reverse transcriptase PCR. J. Clin. Microbiol. **36**:1070–1073.
4. **Ding M, et al.** 2011. An optimized sensitive method for quantitation of DNA/RNA viruses in heparinized and cyropreserved plasma. J. Virol. Methods **176**:1–8.
5. **Ganesan A, et al.** 2010. Immunologic and virologic events in early HIV infection predict subsequent rate of progression. J. Infect. Dis. **201**:272–284.
6. **Holodniy M, et al.** 1991. Inhibition of human immunodeficiency virus gene amplification by heparin. J. Clin. Microbiol. **29**:676–679.
7. **Imai H, Yamada O, Morita S, Suehiro S, Kurimura T.** 1992. Detection of HIV-1 RNA in heparinized plasma of HIV-1 seropositive individuals. J. Virol. Methods **36**:181–184.
8. **Lazarus LH, Kitron N.** 1974. Inhibition and dissociation of mammalian polymeric DNA polymerase by heparin. Arch. Biochem. Biophys. **164**: 414–419.
9. **Lew J, et al.** 1998. Determinations of levels of human immunodeficiency virus type 1 RNA in plasma: reassessment of parameters affecting assay outcome. TUBE Meeting Workshop Attendees. Technology Utilization for HIV-1 Blood Evaluation and Standardization in Pediatrics. J. Clin. Microbiol. **36**:1471–1479.
10. **Pelak K, et al.** 2010. Host determinants of HIV-1 control in African Americans. J. Infect. Dis. **201**:1141–1149.
11. **Roche Molecular Systems, Inc.** 2007. COBAS AmpliPrep/COBAS TaqMan HIV-1 test package insert, version 2.0. Doc Rev. 1.0. Roche Molecular Systems, Inc, Branchburg, NJ.
12. **Rodriguez RJ, et al.** 1997. Comparison of serum and plasma viral RNA measurements in primary and chronic human immunodeficiency virus type 1 infection. J. Acquir. Immune Defic. Syndr. Hum. Retrovirol. **15**:49–53.
13. **Silverberg MJ, et al.** 2006. Effectiveness of highly-active antiretroviral therapy by race/ethnicity. AIDS **20**:1531–1538.
14. **Taylor AC.** 1997. Titration of heparinase for removal of the PCR-inhibitory effect of heparin in DNA samples. Mol. Ecol. **6**:383–385.
15. **Weintrob AC, et al.** 2009. Virologic response differences between African Americans and European Americans initiating highly active antiretroviral therapy with equal access to care. J. Acquir. Immune Defic. Syndr. **52**:574–580.
16. **Witt DJ, Kemper M.** 1999. Techniques for the evaluation of nucleic acid amplification technology performance with specimens containing interfering substances: efficacy of boom methodology for extraction of HIV-1 RNA. J. Virol. Methods **79**:97–111.